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BIOCHEMICAL AND PHARMACOLOGICAL CHARACTERISATION OF SR141716A, THE FIRST POTENT AND SELECTIVE BRAIN CANNABINOID RECEPTOR ANTAGONIST

Murielle Rinaldi-Carmona*, Francis Barth, Michel Héaulme, Richard Alonso, David Shire, Christian Congy, Philippe Soubrié, Jean-Claude Brelière and Gérard Le Fur

Sanofi Recherche, 371 Rue du Professeur Blayac, 34184 Montpellier Cedex 04 (France).

Summary

SR141716A is a selective, potent and orally active antagonist of the brain cannabinoid receptor with a long duration of action. This compound shows high affinity for the central cannabinoid receptor (K_i=2 nM), displays low affinity for the peripheral cannabinoid receptor (K_i>1000 nM). In vitro, SR141716A antagonizes the inhibitory effects of cannabinoid receptor agonists on both mouse vas deferens contractions and dopamine-stimulated adenylyl cyclase activities in rat brain membranes. After oral administration SR141716A totally inhibited the ex vivo [^{3}H]-CP55,940 binding to cerebral membranes with a ED_{50} value of 3.5 mg/kg. Furthermore SR141716A antagonizes the classical pharmacological responses elicited by cannabinoid receptor agonists. In addition, SR141716A reverses the inhibitory effect of WIN55212-2 on isoniazid-induced elevation of cGMP in rat cerebellum.This compound will provide a powerful tool for studying the in vivo functions of the anandamide/cannabinoid system.

Key Words: cannabinoid receptor, receptor antagonist, cAMP, cGMP

Recent reports have shown that Δ^9-THC, the major psychoactive component of cannabis, as well as the putative endogenous ligand for the cannabinoid receptor, anandamide (1) mediate their cellular effects through a specific G protein-coupled receptor in the brain (2). This receptor, designated CB1, recently cloned both in rat (3) and human (4) is also found but in lower abundance in some peripheral tissues (5, 6). A novel type of cannabinoid receptor, designated CB2, has been recently described (7). The CB2 subtype seems to be expressed in immune tissue and may be involved in cannabinoid-mediated immune response, but it appears not be expressed in the brain. This paper introduces SR141716A (Fig. 1) as a novel, highly potent, selective and orally active antagonist for CB1 receptor which is found in the brain(8).

* correspondence

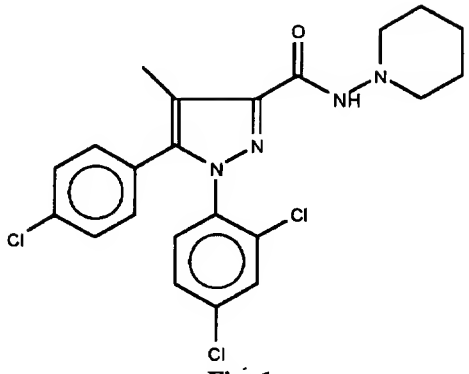

Fig. 1
Structure of SR141716A.
[N-(piperidin-1-yl)-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide hydrochloride]

Methods

Materials. Dopamine (DA) and isoniazid were from Sigma. [^{3}H]-CP55,940 (111.9 Ci/mmol) was from New-England Nuclear Corporation. Kits for cAMP and cGMP assays were from Amersham. CP55,940 was generously provided by Pfizer. WIN55212-2 was purchased from RBI. SR141716A and anandamide were synthesised at Sanofi Recherche. For in vitro assays drugs were dissolved either in ethanol (10^{-3} M) or dimethyl sulfoxide (10^{-2} M).

Animals. Male Sprague Dawley rats (~220 g), male and female mice (CD1~20 g) were obtained from Charles River (France) and used for in vitro, in vivo studies and turning behavior, respectively. Male Swiss mice (~30 g) were obtained from CERJ, Le Genet S^t Isle (France) and used for vas deferens preparations. Male mice (Of1,~25 g) and rats (Ofa,~120 g) were obtained from Iffa Credo (France) and used for the others psychopharmacological tests.

Drug preparation and administration. For in vivo experiments, drugs were dissolved in two drops of Tween 80, diluted in distilled water and administered intraperitoneal (i.p.) or per os (p.o.) in a volume of 20 ml/kg to mice or 5 ml/kg to rats. Intrastriatal injection of cannabinoid agonists was made in a volume of 1 µl. For the intravenous (i.v.) injection WIN55212-2 dissolved in two drops of Tween 80 was diluted in saline solution. Doses are expressed as the salt.

Membrane preparations. Membranes were prepared from rat brain as in (9), from rat spleen as in (5) and from chinese hamster ovary (CHO) cells, expressing either CB1 or CB2, as in (10).

In vitro binding assays. Experiments were carried out with [^{3}H]-CP55,940 for 1 h at 30° C as described in (8). Non-specific binding was determined in the presence of 1 µM CP55,940.

Ex vivo binding assays. Drugs or vehicle were administered i.p. or p.o. to mice before they were killed by decapitation. The brain (without the cerebellum) or the spleen were removed and homogenized in binding buffer and assays performed as described in (8).

Mouse vas deferens preparations. Assays were performed as described in (see 8).WIN55212-2 was added once the contractile responses to electrical stimulation were reproducible. Preparations were exposed to cumulative increasing concentrations of agonists either in the absence (control) or

in the presence of SR141716A added at a given concentration 60 min before the first agonist concentration.

Adenylyl cyclase assays. DA-stimulated adenylyl cyclase activity was carried out in rat striatal membranes as in (11). Sulpiride (10^{-5} M) was included in the medium. The cAMP concentration was determined by radioimmunoassays.

cGMP determination in rat cerebellum. Cerebellar cGMP levels were determined as described in (12). cGMP level was measured by radioimmunoassay.

Psychopharmacological tests, in rodents. Behavioural responses induced by the intravenous (i.v.) injection of WIN55212-2 were assessed either fifteen minutes [tail flick (13), ring-immobility (14) or thirty minutes (hypothermia (15)] after the administration of SR141716A. Pop corn effects in mice and barrel rotations in rats were analyzed as described in (16) and (17), respectively. Turning behavior induced by intrastriatal injection (18) of cannabinoid agonists were performed thirty minutes after the i.p. administration of SR141716A.

Data analysis. Data from binding were analyzed using a non-linear least-squares method. K_d values of 0.23 ± 0.04 nM (brain) and 0.65 ± 0.06 nM (CB1) were used to determine K_i values, respectively.Data from receptor antagonism studies were analyzed by the Student *t* test.

Results

Interaction of SR141716A with brain cannabinoid receptors in vitro. As shown in Fig. 2A, the specific binding of [3H]-CP55,940 to its high affinity receptor in rat brain synaptosomal membranes was totally displaced by SR141716A in a concentration-dependent manner with a K_i value of 1.98 ± 0.36 nM (n=4). In contrast, SR141716A did not interact with the cannabinoid receptor expressed in rat spleen (38.6 ± 2.5 % inhibition at 10^{-6} M, n=3). Furthermore, as shown in Fig. 2B in CHO cell membranes expressing human CB1, SR141716A was a potent inhibitor of [^{3}H]-CP55,940 binding sites with a K_i value of 5.6 ± 0.5 nM (n=4) displaying only a very low affinity for membranes from CHO cells expressing human CB2 (38.7 ± 0.8 % inhibition at 10–6 M, n=4). These results show that SR141716A is selective for CB1 versus CB2 and interacts with the cloned human cannabinoid receptor. In addition, [^{3}H]-CP55,940 saturation binding experiments performed in rat brain membranes showed that SR141716A is a competitive ligand for CB1 (not shown).

Cannabinoid receptor antagonism properties of SR141716A in vitro. As shown in Fig.3, in the mouse vas deferens WIN55212-2 induced a concentration-dependent inhibition of the twitch contractions with a pD_2 value of 8.93 ± 0.05 (n=5). SR141716A competitively antagonized the inhibitory effects of WIN55212-2 with a pA_2 value 8.85 ± 0.05. This result confirms previous data (8) showing that it behaved as a competitive antagonist versus the synthetic cannabinoid agonists CP55,940 and the putative endogenous ligand anandamide.

The stimulation of the adenylyl cyclase activity by DA (10^{-4} M) in striatal membranes was inhibited by WIN55212-2 with a EC_{50} value of 3.2 ± 1.1 µM (n=5). As shown in Fig. 4, SR141716A which had no effect by itself totally reversed the inhibition evoked by WIN55212-2 (10^{-5} M) with a IC_{50} value of 16 ± 4 nM (n=6). This finding was closed to previous data (8) showing that SR141716A is able to antagonize WIN55212-2-induced forskolin-stimulated adenylyl cyclase inhibition in rat substantia nigra membranes. Taken together, these in vitro results indicate that SR141716A is a potent, competitive and selective antagonist of the brain cannabinoid receptor.

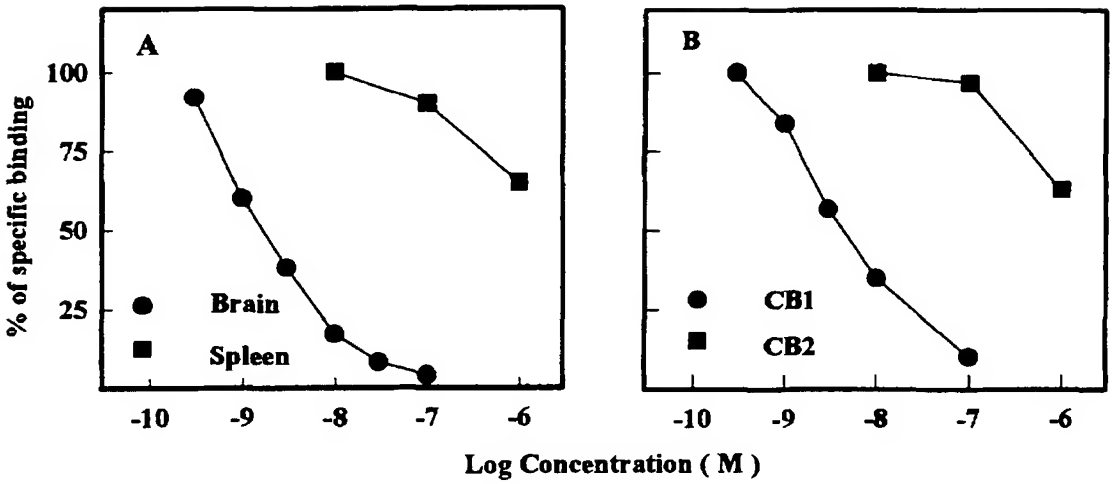

Fig. 2

Inhibition of [^{3}H]-CP55,940 binding to rat (A) and CHO (B) membranes by SR141716A. Assays were carried out as described under "Methods" using 0.1 nM [^{3}H]-CP55,940 and increasing concentrations of SR141716A. Data are from one experiment out of 4 and are expressed as the percentage of binding in the absence of competitor.

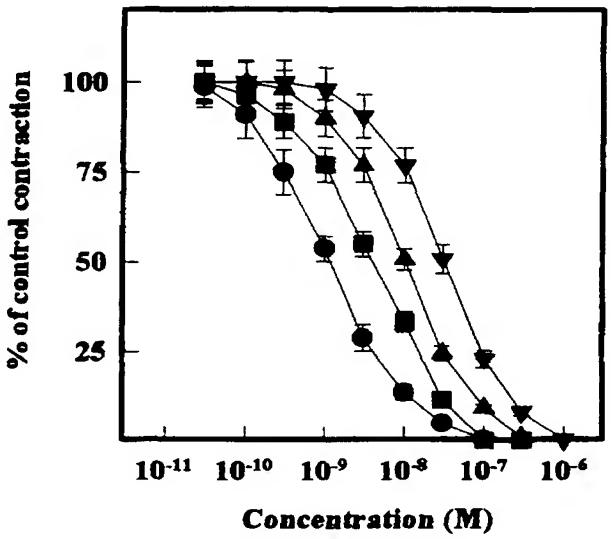

Fig. 3

Cumulative concentration-response curves for WIN55212-2 on the amplitude of twitch contractions elicited by electrical field stimulation of the mouse vas deferens obtained in the absence (●)(control) and in the presence of SR141716A at $3x10^{-9}$ M (■), 10^{-8} M (▲) and $3x10^{-8}$ M (▼). Assays were performed as described under "Methods". Data are expressed as a percentage of control values after incubation with SR141716A. Each point is the mean value ± S.E.M of 6 determinations.

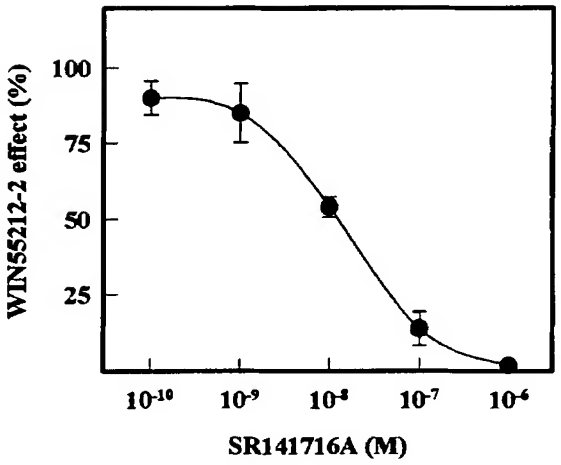

Fig. 4

Reversal by SR141716A of WIN55212-2 induced inhibition of DA-sensitive adenylyl cyclase activity. Assays were performed as described under "Methods". Values are means ± S.E.M. from 6 experiments performed in triplicate. Data are expressed in percentage of WIN55212-2 effect at 10^{-5} M in the striatum (DA alone, 91 ±11 pmol/mg/min., + WIN55212-2, 40.6 ± 3.6 pmol/mg/min)

In vivo interaction of SR141716A with central cannabinoid receptors. After i.p. or po. administration, SR141716A totally inhibited the specific binding of [^{3}H]-CP55,940, measured ex vivo, to cerebral membranes in a dose–dependent manner with median effective dose values (ED_{50}) of 1.12 ± 0.21 mg/kg and 3.60 ± 0.37 mg/kg (n=3), respectively. In contrast, SR141716A did not interact with the cannabinoid receptor expressed in mouse spleen, (20% inhibition at 10 mg/kg, i.p.). As shown in Fig. 5, at 10 mg/kg 50 % of the brain cannabinoid receptor remained significantly occupied during 8 h after oral administration of SR141716A. These results indicate that SR141716A is able to cross the blood–brain barrier, has a long duration of action and they confirm its specificity for the brain cannabinoid receptor.

In vivo antagonism of SR141716A at the central cannabinoid receptor. As previously shown for levonantradol (12), WIN55212-2 administered i.p.(30 min.) is able to inhibit dose-dependently the elevation of rat cerebellar cGMP levels caused by the i.p. (45 min.) administration of isoniazid, with an ID_{50} value of 0.2 ± 0.04 mg/kg (n=3). Pretreatment of animals with SR141716A (30 minutes, i.p.) totally reversed this inhibition with a ED_{50} value of 0.45 ± 0.08 mg/kg (n=3).

The in vivo antagonism of SR141716A for the brain cannabinoid receptor was investigated in several animal models classically used to study cannabinoid drug effects. SR141716A administered i.p. or p.o potently and dose-dependently antagonisd the responses elicited by WIN55212-2 in all evaluation tests, including : hypothermia, antinociception, ring-immobility, popcorn effect, barrels rotations, with a ID_{50} value ranging from 0.1 to 1.7 mg/kg (8). Furthermore, the turning behaviour induced by intrastriatal injection of cannabinoid receptor agonists including anandamide was antagonised by SR141716A with an ED_{50} value ranging from 0.15 to 0.30 mg/kg after i.p. administration. These results show that SR141716A is a functional antagonist of the brain cannabinoid receptor (CB1) with a good oral bioavailability and a long duration of action.

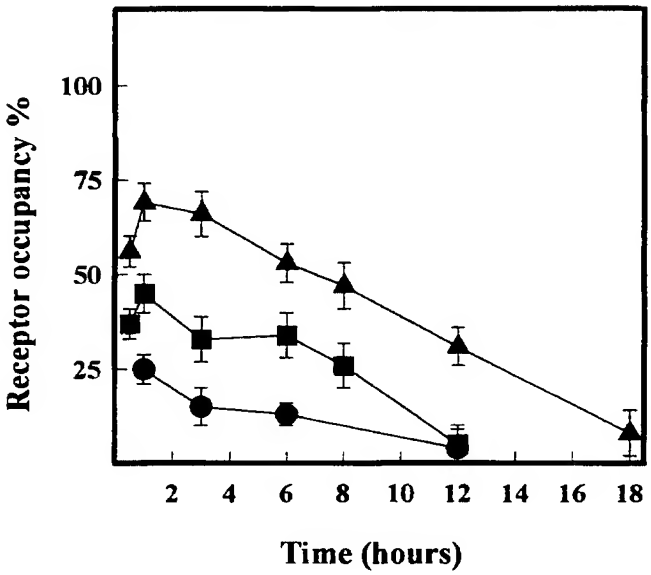

Fig. 5

Time course of the brain cannabinoid receptor occupancy by SR141716A after p.o. administration. Mice were administered with 1 (●), 3 (■) or 10 (▲) mg/kg of SR141716A. They were sacrificed at different times after these administrations. Then binding studies were performed as described under "Methods". Data are means ± S.E.M. of six values obtained from three animals. They are expressed as percentage of inhibition of the specific binding in tissues of untreated mice.

Discussion

In conclusion, SR141716A, appears to be a potent, selective and orally active cannabinoid receptor antagonist with a 1000 fold higher affinity for CB1 than for CB2 receptors. Functional in vitro and in vivo studies show that SR141716A is able to antagonise the pharmacological effects induced by both the cannabinoid receptor agonists, CP55,940, WIN55212-2, and the putative endogenous receptor ligand anandamide. Finally, in vivo studies showing the reversal by SR141716A of WIN55212-2-induced inhibition of cGMP levels increased by isoniazid in rat cerebellum strongly suggests the involvement of CB1 in the control of glutamate release. This assumption is supported by the fact that WIN55212-2 did not affect the increase of cGMP produced either by stimulation of climbing fibers (harmalin) or postsynaptic glutaminergic receptor (NMDA). Thus SR141716A may be considered as a useful tool to elucidate the respective physiological or pathophysiological roles of CB1 versus CB2 cannabinoid receptors. It further support the proposal that anandamide may be a good candidate as an endogenous CB1 ligand. It is expected that this discovery will provide a new tool to better understand the mechanisms by which marijuana produces its pharmacological effects and to develop potential therapeutic agents.

References

1. W. A. DEVANE, L.HANUS, A BREUER, R.G. PERTWEE, L.A. STEVENSON, G. GRIFFIN, D. GIBSON, A. MANDELBAUM, A. ETINGER and R. MECHOULAM, Science 258 1946-1949 (1992).

2. W A. DEVANE, F. A. DYSARZ, M. R. JOHNSON, L. S. MELVIN and A. C. HOWLETT, Molec. Pharmacol. 34 605-613 (1988).
3. L. A. MATSUDA, S.J. LOLAIT, B.J. BROWNSTEIN, A.C. YOUNG and T.L. BONNER, Nature 346 561-564 (1990).
4. C. M. GERARD, C. MOLLEREAU, G. VASSART and M. PARMENTIER, Biochem. J. 279 129-134 (1991).
5. M. BOUABOULA, M. RINALDI, P. CARAYON, C. CARILLON, B. DELPECH, D. SHIRE, G. LE FUR and P. CASELLAS, Eur. J. Biol. Chem. 214, 173-180 (1993).
6. N. E. KAMINSKI, M. ABOOD, F.K. KESSLER, B.R. MARTIN and A.R. SCHATZ, Molec. Pharmacol. 42 736-742 (1992).
7. S. MUNRO, K. L. THOMAS and M. ABU-SHAAR, Nature 365 61-65 (1993).
8. M. RINALDI-CARMONA, F. BARTH, M. HEAULME, D. SHIRE, B. CALANDRA, C. CONGY, S. MARTINEZ, J. MARIUANI, G. NELIAT, D. CAPUT, P. FERRARA, P. SOUBRIE, J-C. BRELIERE and G. LE FUR, FEBS Letters. 350 240-244 (1994).
9. J. P. ABITA, R. CHICHEPORTICHE, H. SCHWEITZ and M. LAZDUNSKI, Biochemistry 16 1838-1843 (1977).
10. M. LANDI, A. BIANCHETTI, T. CROCI and L. MANARA, Biochem. Pharmac. 44 665-672 (1992).
11. J. LUTHMAN, E. LINDQVIST, D. YOUNG and R. COWBURN, Exp. Brain. Res. 83 85-95 (1990).
12. J. P. LEADER, B. KENNETH KOE and A. WEISSMAN, J. Clin. Pharmacol. 21 262S-270S (1981).
13. R. G PERTWEE, Br. J. Pharmac. 46 753-763 (1972).
14. F. E. AMOUR and D. L. SMITH, J. Pharmac. Exp. Ther.72 74-79 (1941).
15. R. G. PERTWEE, Marijuana. 84 263-277, IRL Press, Oxford (1985).
16. W. L. DEWEY, Pharmac.Rev. 38 151-178 (1988).
17. M. L. COHN, Brain Res .96 138-141 (1975).
18. P. WORMS, J. MARTINEZ, C. BRIET, B. CASTRO and K. BIZIERE, Eur. J. Pharmacol. 121 395-401 (1986).